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Influence of the Feeding Pattern on the Glucose Metabolism of *Arthrobacter sp.* and *Sphaerotilus natans*, Growing in Chemostat Culture, Simulating Activated Sludge Bulking

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Summary. Changing from a continuous feeding to an intermittent feeding selects for floc-forming micro-organisms in activated sludge systems. Previous research stressed the importance of the substrate uptake rate. In order to explain this shift in population the uptake kinetics of pure cultures of Sphaerotilus natans (filament) and Arthrobacter (floc-former) were examined with glucose as C-source.

Batch experiments indicated a severe decrease in uptake capacity at the end of the declining growth phase.

The results with continuously and intermittently fed chemostats indicated a profound influence of periodic feeding on the metabolism of pure cultures; steady state dry weights were lower, substrate uptake over-capacity was larger, more over-flow metabolites were produced. Higher K_m values for substrate uptake were recorded and more reserve materials were built. More important, the over-capacity in I-fed cultures of the floc-forming micro-organism was larger than for the filamentous bacterium. In ecological perspective this may explain the selection of a floc-forming biomass in activated sludge systems with temporary higher BOD-concentrations or operating in plug-flow.

Introduction

Activated sludge is a complex mixed population of bacteria and protozoa. The composition of this flora is influenced by many environmental factors (temperature, wastewater characteristics, sludge loading, oxygen supply). Recently it was put forward that the feeding pattern had a profound influence on the species composition. For pure substrates and for industrial waste streams it was found that continuously (C) fed activated sludge systems produced a microbial population with a high proportion of filamentous bacteria causing bulking problems. Intermittent (I) feeding suppressed their growth (Houtmeyers et al. 1980; Verachtert et al. 1980; Van den Eynde et al. 1982). I-feeding is characterized by a sequence of an exogenous phase (with substrate in solution just after the feeding pulse) and an endogenous phase (without measurable amounts of substrate in solution). Under these conditions the micro-organisms are growing at alternatively high and low substrate concentrations. In continuously fed systems the substrate concentration is always low. It was found that the substrate uptake rate in the I-fed cultures is higher. This fact is probably a consequence, not only of the feeding pattern itself but also of the different populations growing in the respective cultures. In order to separate both influences it is necessary to study the uptake rate of pure cultures, both under C- and I-feeding.

Due to the limited permeability of the microbial membrane, uptake of nutrients is mediated by active proteins. In group translocase the substrates are chemically modified during uptake, in substrate translocation the substrates are transported without modification. Energy has to be supplied if substrates are taken up against a concentration gradient. For all these systems active site kinetics are believed to obey the Michaelis-Menten equation and are thus characterised by a q_{max} (maximal specific substrate uptake rate) and a K_m-value.

In this article the substrate uptake rate and the way it is influenced by the feeding pattern are studied. Pure cultures of a floc-forming (*Arthrobacter sp.*) and a filamentous bacterium (*Sphaerotilus natans*) are examined. A metabolic explanation for the selective stress of the feeding pattern is put forward.

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Materials and Methods

Isolation of the Micro-Organisms. Two bench scale activated sludge units (Houtmeyers et al. 1980) were inoculated with sludge originating from the purification system of a dairy plant. One was C-fed and the other I-fed with a glucose-mineral medium; the sludge loading was set at 0.3 g COD · g $MLSS^{-1}$ · day^{-1} (COD is the Chemical Oxygen Demand and MLSS stands for Mixed Liquor Suspended Solids). The filamentous population growing in the C-fed system was subjected to the isolation procedure for filaments of Van Veen (1973). On *his* "I"-medium a pure culture was isolated showing ensheated filaments with false branching, Gram negativity, cells clearly separated with crosswalls visible, accumulation of poly-β-hydroxybutyric acid in rich glucose media and requirement of vitamine B12 for growth with inorganic nitrogen. According to many authors (Bergey's Manual 1974; Van Veen 1973; Eikelboom 1981) these properties are sufficient to identify it as *Sphaerotilus natans*.

From the I-fed culture, free of extending filaments, a pure culture was isolated showing a changing morphology during the growth cycle, with young cells being catalase positive, strict aerobic, not degrading cellulose and not needing vitamins with inorganic nitrogen. This species was tentatively identified as belonging to the genus *Arthobacter*

Table 1. Media for pure cultures

Component and concentration	Medium			
	A	B	C	D
Glucose (2 g · l^{-1})	+	+	+	+
$(NH_4)_2SO_4$ (2 g · l^{-1})	+	+	−	+
Tryptone (1 g · l^{-1})	−	−	+	−
Methionine (100 mg · l^{-1})	−	−	−	+
Vitamin mix[a] (10 ml · l^{-1})	−	+	−	−
K_2HPO_4 (3.2 g · l^{-1})	+	+	+	+
Mineral mix[b] (10 ml · l^{-1})	+	+	+	+
pH = 7.2	+	+	+	+

[a] Composition of the vitamin mix (g · l^{-1}) vitamine B_{12} 10^{-3}, thiamine 4.10^{-2}

[b] Composition of the mineral mix (g · l^{-1}) $MgSO_4 \cdot 7\ H_2O$ 5, $CaCl_2 \cdot 6\ H_2O$ 1, NaCl 1, $FeCl_3$ 0.25, $MnSO_4 \cdot H_2O$ 1

Media Used. The growth media are listed in Table 1.

Growth Conditions. Batch cultures in 500 ml erlenmeyer flasks (active volume of 150 ml) were incubated on a transversal shaker (100 cycles · min^{-1}) at 28° C, resulting in an oxygen transfer of 22 mmol · h^{-1} · l^{-1} for 150 ml water in a 500 ml flask.

In order to obtain continuous cultures, organisms were grown in a 1 l chemostat (Modular Fermentor A. Gallenkamp, London) with a 600 ml working volume. For continuous feeding the feed was pumped continuously, through a drip-feed, into the fermentation vessel. Intermittent feeding was achieved by collecting the feed in a sterile intermediate vessel and by introducing its content into the fermentor once every hour, by the controlled opening of an autoclavable electromagnetic valve. The complete unit was autoclaved with a few drops of water in the fermentation vessel and with the electrical parts of the valve uncoupled. After cooling the feeding pump was started and the vessel was filled with sterile medium. The temperature was set at 28°C. Aeration and mixing were switched on and the fermentor was kept untouched for 48 h to check its sterility. The fermentor was then inoculated and batch growth could proceed. When good growth was visible the feeding and harvest pumps were started and the desired dilution rate was set.

Analytical Techniques. Glucose was assayed according to the method of Nelson (1944). Concentrations lower than 20 mg · l^{-1} were assayed enzymatically with the glucose oxidase-peroxidase system. The ABTS chromogen [2.2'-Azino-di-(3-ethylbenzthiazolin-sulfonate[6])] was used instead of o-dianisidine and determined at 415 nm on a Perkin Elmer 550 S spectrophotometer.

Bacterial dry weight was determined by the procedure of Herbert et al. (1971). Polysaccharide reserve materials were isolated according to Slock and Stahly (1974) and purified according to Carrol et al. (1956). Qualitative estimations of the carbohydrate content of whole cells were made using the anthron method. Poly-β-hydroxybutyric acid was estimated gaschromatographically according to Braunegg et al. (1978) with some modifications. A 2 m column, with 4 mm internal diameter, was filled with 10% Carbowax 20 M on Chromosorb W (80–100 mesh) and isothermal conditions were used (115° C). Injection and detection blocks were kept at 240° C.

Glucose uptake kinetics were determined on samples taken from the fermentor. After addition of a cell saturating dose of

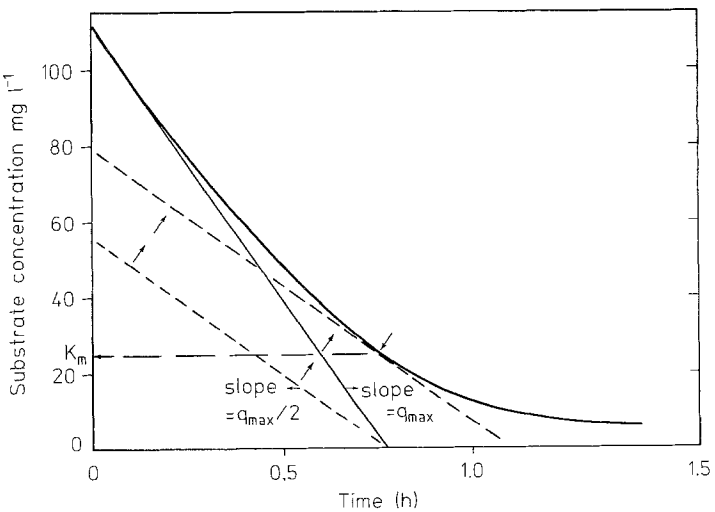

Fig. 1. Uptake curve and graphical method for K_m determination

glucose the decrease of the glucose concentration during incubation was followed by repeated sampling (5 min intervals). The slope of the uptake line represents the maximum uptake rate. Dividing by the dry weight gives the specific maximal uptake rate q_{max} (mg glucose · g^{-1} · min^{-1}). K_m-values for glucose uptake were estimated with three methods; the symbol K_m is used to indicate that the value is different from, though related to K_s for growth (Appendix 1).

Method 1; Substrate uptake was followed until the rate started to decrease. Drawing the tangent with slope $q_{max}/2$, K_m can be readily found on the Y-axis (Fig. 1).

Method 2; Another technique in which the biomass is assumed to be constant is the method of Counotte and Prins (1979). The points of the uptake curve (Fig. 1.) are used as data and a value of q_{max} and K_m is found by a graphical procedure.

Method 3; In this indirect method K_m is determined by way of the substrate respiration. To bacterial suspensions placed in an Oxygraph, fitted with a Clark oxygen electrode (Yellow Springs Instrument Model 54A, Ohio, USA), minor amounts of substrate are added. The initial respiration rates are corrected for the endogenous respiration and data-couples (rate-substrate concentration) are plotted according to Lineweaver-Burk. Curves were considered as straight lines when a correlation coefficient of 0.97 was found for at least seven points.

Results

A. Batch Culture Data

Preliminary batch experiments were performed to study the growth and substrate uptake behaviour. *Arthrobacter* grew well in medium A and logarithmic growth was observed. μ_{max} was calculated using dry weight ($\mu_{max\,X}$) and cell number ($\mu_{max\,N}$) data. The Yield (Y) was expressed on a weight/weight base. The following formulas were used:

$$\frac{1}{N}\cdot\frac{dN}{dt}=\mu_{max\,N}\quad h^{-1}, \tag{1}$$

$$\frac{1}{X}\cdot\frac{dX}{dt}=\mu_{max\,X}\quad h^{-1}, \tag{2}$$

$$\frac{\sum\frac{X_t-X_0}{S_0-S_t}}{n}=Y\quad g\ biomass\cdot g\ substrate^{-1}, \tag{3}$$

with N: the cell number, X: the cell dry weight, S: the substrate concentration, n: the number of samplings, index t: the elapsed time, index 0: the condition at zero time.

$$\mu=\mu_{max}\cdot\frac{S}{K_s+S}\quad h^{-1}, \tag{4}$$

$$\frac{dX}{dt}=-Y\cdot\frac{dS}{dt}\quad g\cdot l^{-1}\cdot time^{-1}. \tag{5}$$

Equations (2), (4), and (5) were solved simultaneously for the (X, S, t) data of a batch growth experiment. The Yield, μ_{max} and K_s for growth can be calculated based on a complete growth curve (Table 2). Gates and Marlar (1968) proposed a graphical solution for this set of equations. It is a trial and error method for findings a line of best fit. In this work the procedure was adapted. On introduction of the Yield value [from Eq. (3)] and an estimated value of the μ_{max}, the set of equations can be solved mathematically for a value of K_s. For each growth state (X, S, t) a value of K_s can be calculated. For each pair (Y, μ_{max}) a single K_s-value is found by averaging over the number of samples. A higher or lower value for the μ_{max} estimation will result in a different K_s-value. The K_s, μ_{max}-combination for which the sum of $(X_{calculated}-X_{experiment})^2$ and the sum of $(S_{calculated}-S_{experiment})^2$ are minimal, is the best estimate for the kinetic parameters. A Fortran program of this procedure was written.

The kinetics of substrate uptake were more thoroughly investigated on non-growing systems. Batch cultures in which the glucose was just exhausted were used. q_{max} was determined according to Method 1 and Method 2 and K_m according to Method 1, 2, and 3. The results are compared in Table 3.

Likewise *Sphaerotilus natans* was cultivated in batch. For growth in mineral glucose medium this filamentous bacterium requires vitamins (Mulder and Van Veen 1962; Okrend and Dondero 1964). Incorporation of vitamin B_{12} and thiamine stimulates the growth. In medium B *Sphaerotilus natans* produced macrosopic pellets in a clear medium. The biomass adhered to the glasswall of the shaken flask and growth was slow.

Typical filamentous growth was obtained in media with an organic N-source. In medium C long filaments protruding from the flocs increased the viscosity of the growth medium. Methionine, in minor amounts could replace the tryptone. All the cells contained refractive globules; cytochemical staining (sudan black) and chemical analyses of the cells proved them to consist of PHB. Polymer contents of upto 50% were recorded with the highest values at the end of the batch growth. Batch growth in medium C showed a diauxic effect; the first phase was prolonged with increasing tryptone concentrations. No diauxic growth was found in Medium D.

Although *Sphaerotilus natans* was growing as a filament logarithmic growth was observed. Growth parameters calculated on the growth curves are recorded in Table 2, and substrate uptake rate of fully grown *Sphaerotilus natans* cultures resulted in q_{max} and K_m-values (Table 3).

Table 2. Kinetic parameters of growth of *Sphaerotilus* and *Arthrobacter* in batch

	μ_{max} N (h^{-1})	μ_{max} X (h^{-1})	Y ($g \cdot g^{-1}$)	K_s[a] ($mg \cdot l^{-1}$)
Arthrobacter				
Medium A	0.29–0.32	0.35–0.38	0.49–0.54	23–29
Sphaerotilus				
Medium C	–	0.13–0.15	0.46–0.53	–
Medium D	–	0.13–0.14	0.47–0.52	–

[a] According to Gates and Marlar (1968)

Table 3. Kinetic parameters of substrate uptake of *Sphaerotilus* and *Arthrobacter* in batch

	Arthrobacter sp.		*Sphaerotilus natans*			
	Medium A		Medium C		Medium D	
	q_{max} ($mg \cdot g^{-1} \cdot min^{-1}$)	K_m ($mg \cdot l^{-1}$)	q_{max} ($mg \cdot g^{-1} \cdot min^{-1}$)	K_m ($mg \cdot l^{-1}$)	q_{max} ($mg \cdot g^{-1} \cdot min^{-1}$)	K_m ($mg \cdot l^{-1}$)
Method 1 (graphical)						
Exp. 1	3.6	18	1.7	15	2.9	16
2	4.7	20	1.9	18	3.1	12
3	3.7	15	1.7	19	3.0	15
4	3.8	16	–	–	–	–
Method 2 (Counotte)						
Exp. 1	4.0	16	1.8	12	3.4	12
2	–	–	2.0	15	3.1	12
3	4.2	19	2.1	16	3.2	14
4	4.1	17	–	–	–	–
Method 3 (Respiration)						
Exp. 1	–	15	–	11	–	8
2	–	16	–	–	–	10
3	–	–	–	9	–	9
4	–	20	–	–	–	–

Discussion. Combining Eqs. (4) and (5) and referring to Appendix 1, the q-value corresponding to the μ_{max} can be calculated. *Arthrobacter* growing with a μ_{max} of 0.36 h^{-1} and having a yield of 0.52 should manifest q_{max} during growth of 11.5 $mg \cdot g^{-1} \cdot min^{-1}$. The same rationale applied to *Sphaerotilus natans* growing with μ_{max} of 0.14 h^{-1} and $Y = 0.49$ should lead to a q_{max} of 4.8 $mg \cdot g^{-1} \cdot min^{-1}$. The values of the kinetic parameters in Table 3 indicate that the q_{max}-values measured on fresh cultures, just as the substrates are exhausted, never reach these high values. *Arthrobacter* cultures show a severe decrease in uptake capacity and also *Sphaerotilus natans* shows some kind of metabolic control. Probably during the decreasing growth phase modifications of the metabolic control are induced. The metabolism may be adapting towards using reserve materials or other endogenous substrates. The higher substrate uptake rate of *Sphaerotilus* in Medium D stresses the importance of methionine in the growth-medium.

Estimation of K_m for non-growing cells (X = constant in Method 1, 2, and 3) resulted in acceptable straight line fittings, thus proving Michaelis-Menten kinetics. Method 1 and the method of Counotte are processing the same data; it is obvious that similar K_m-values were found. In contrast with these methods, where K_m values were estimated at the end of a glucose uptake experiment. A method 3 calculates K_m on initial respiration rates, after addition of only small amounts of glucose. Since this method is imposing the least stress on the bacterial cells, it will probably give the best estimate for K_m, accepting that the uptake of the substrates is the rate limiting step. By this procedure often lower values were found.

The K_m values for both species are low, with the highest affinity for *Sphaerotilus natans*. Species with a lower q_{max} (e. g., *Sphaerotilus natans*) should manifest a lower K_m-value in order to compete effectively (manifesting equal rates) with species with a higher q_{max} (Fig. 2).

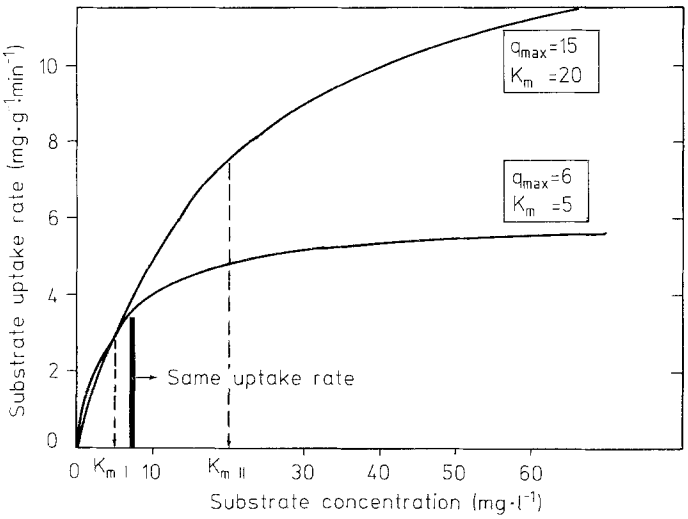

Fig. 2. Rate versus substrate concentration curves, showing a region of competitive uptake rate

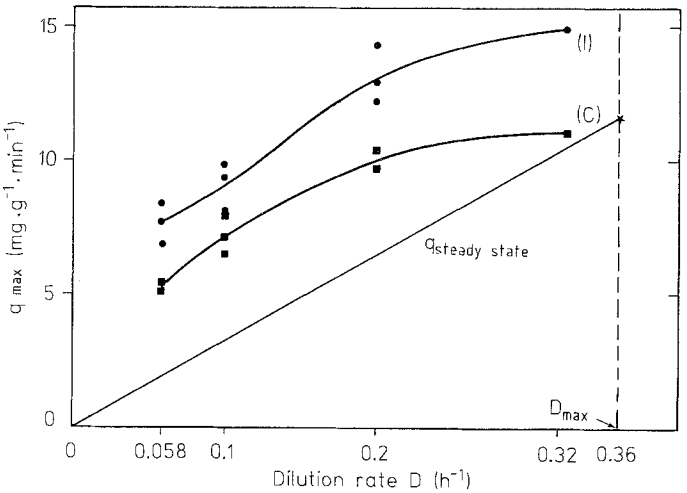

Fig. 3. Comparing q_{max} and $q_{steady\ state}$ for *Arthrobacter sp.* at different D-values in chemostats with I-feeding (●) and C-feeding (■)

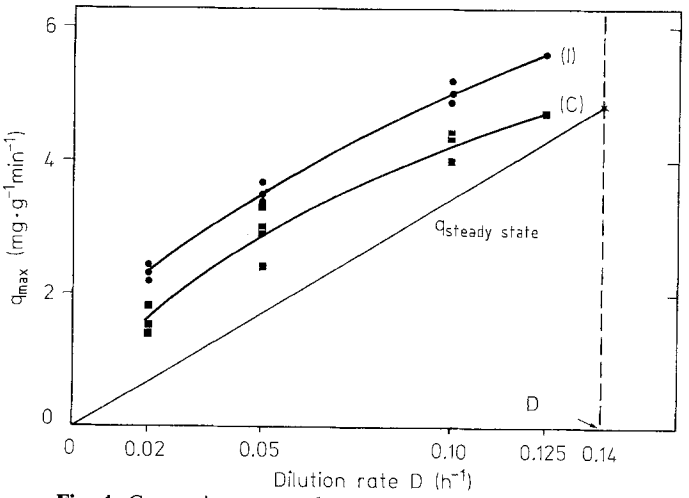

Fig. 4. Comparing q_{max} and $q_{steady\ state}$ for *Sphaerotilus natans* at different D-values in chemostats with I-feeding (●) and C-feeding (■)

After batch growth the uptake mechanisms have been modified and continuous cultures were studied.

B. Continuous Culture Data

Comparing continuously (CC) and intermittently (IC) fed continuous cultures, it is clear that a true „steady state" is existing only in the first type; the substrates introduced in a continuous way are taken up immediately. Time is *no* state variable of these systems. On the other hand it *is* in the IC-chemostats. No steady state exists in this case; only a repetition of substrate gradients in time, separated by endogenous phases. It is expected that this stress on the growing cultures may influence the uptake mechanism and uptake kinetics.

IC and CC-chemostats of *Arthrobacter*, using Medium A, were examined at different dilution rates. Medium D was used to study *Sphaerotilus natans*. *Arthrobacter* cultures were lemmon yellow and produced small flocs. The *Sphaerotilus* cultures were composed of a lattice work of long threads. The sheaths and their water binding capacity were rendering the cultures highly viscous. Aeration and stirring were difficult.

Substrate uptake kinetics were studied and the values of q_{max} and K_m are recorded in Tables 4 and 5 and in Figs. 3 and 4. From the IC-cultures samples were taken 3 min before a new feeding pulse.

General Discussion

In accordance with the general theory of the chemostat (Herbert et al. 1956), dry weight values increased with the dilution rate. Residual substrate concentration was always low, indicating low true K_s-values; estimated lower than 5 mg · l^{-1} for both microorganisms.

At the highest growth rates, apart from a low concentration of glucose, considerable amounts of oxidizable material were found in the culture media. Their appearance coincided with a decrease of the pH, indicating that the compounds leaking out of the cells are organic acids. Under slightly different conditions Neijssel (Neijssel and Tempest 1975) identified a number of so called "overflow metabolites"; pyruvate, 2-oxoglutarate, acetate, gluconic acid, 2-keto-gluconic acid and succinate were found in glucose sufficient chemostats.

For all fermentations and dilution rates studied, q_{max}-values were higher than the corresponding steady state uptake rates, repuired to assimilate the

Table 4. Continuous culture data of *Arthrobacter*

Day	D (h^{-1})	X ($g \cdot l^{-1}$)	S ($mg \cdot l^{-1}$)	pH	Method 1		Method 3
					q_{max} ($mg \cdot g^{-1} \cdot min^{-1}$)	K_m ($mg \cdot l^{-1}$)	K_m ($mg \cdot l^{-1}$)
Arthrobacter – continuous feeding – Medium A – 28° C							
3	0.058	0.91	< 2	7.0	5.1	7	2
6	0.058	0.94	< 2	6.9	5.4	9	3
9	0.1	0.96	< 2	6.8	6.5	6	4
12	0.1	0.95	< 2	6.8	7.1	6	–
15	0.1	0.97	< 2	6.8	7.9	8	4
18	0.2	0.96	5 (30)	6.7	10.4	10	7
21	0.2	0.99	3 (30)	6.7	9.7	9	6
24	0.32	1.0	8 (110)	6.7	11.0	10	5
Arthrobacter – intermittent feeding – Medium A – 28° C							
3	0.042	0.83	< 2	6.6	6.9	15	18
6	0.042	0.86	< 2	6.5	7.7	14	13
9	0.042	0.87	< 2	6.4	8.3	12	–
12	0.1	0.92	< 2	6.5	8.0	17	19
15	0.1	0.93	< 2	6.6	9.8	20	20
18	0.1	0.93	< 2	6.6	9.4	21	16
21	0.2	0.96	6 (50)	6.4	12.2	21	25
24	0.2	0.95	9 (70)	6.5	14.4	23	21
27	0.2	0.96	7 (75)	6.4	12.9	23	24
30	0.32	0.98	31 (160)	6.3	14.9	20	21

Note: S-values between brackets are COD-values ($mg \cdot l^{-1}$) < means lower than

Table 5. Continuous culture data of *Sphaerotilus*

Day	D (h^{-1})	X ($g \cdot l^{-1}$)	S ($mg \cdot l^{-1}$)	pH	Method 1		Method 3
					q_{max} ($mg \cdot g^{-1} \cdot min^{-1}$)	K_m ($mg \cdot l^{-1}$)	K_m ($mg \cdot l^{-1}$)
Sphaerotilus – continuous feeding – Medium D – 28° C							
3	0.02	0.81	< 2	7.0	1.8	9	6
6	0.02	0.79	< 2	7.0	1.4	12	8
9	0.02	0.84	< 2	7.0	1.5	10	5
12	0.05	0.89	< 2	6.7	2.4	9	–
15	0.05	0.90	< 2	6.9	2.9	16	6
18	0.05	0.92	< 2	6.9	3.0	12	–
21	0.05	0.92	3	6.8	3.3	10	5
24	0.1	0.92	6	6.7	4.3	14	–
26	0.1	0.94	5 (35)	6.9	4.0	9	8
28	0.1	0.95	8 (42)	6.8	4.4	11	8
30	0.125	0.95	29 (180)	6.7	4.7	10	7
Sphaerotilus – intermittent feeding – Medium D – 28° C							
3	0.02	0.76	< 2	6.9	2.2	15	8
6	0.02	0.80	< 2	6.9	2.4	12	6
9	0.02	0.79	< 2	6.9	2.4	17	7
12	0.05	0.86	< 2	6.7	3.4	13	11
15	0.05	0.86	4	6.7	3.7	15	11
18	0.05	0.87	< 2	6.7	3.5	17	10
21	0.1	0.91	4 (80)	6.6	4.9	15	14
24	0.1	0.90	6 (80)	6.6	5.0	21	12
27	0.1	0.93	7 (90)	6.7	5.2	19	13
30	0.125	0.93	34 (195)	6.6	5.6	19	15

Note: S-values between brackets are COD-values ($mg \cdot l^{-1}$) < means lower than

substrates pumped into the vessel (Figs. 3 and 4). With *Klebsiella aerogenes* O'Brien (O'Brien et al. 1980) found the same behaviour. These results indicate that even continuously fed cells in a chemostat possess a certain "overcapacity" for substrate uptake. This would mean that there is no direct coupling between the energy yielding reactions and the energy consuming reactions. The overcapacity of the cells is only manifest when a pulse dose of substrate is added to the cultures. Where the continuously fed cells were given only one peak dose (during the determination of q_{max}), intermittent cultures were repeatedly subjected to glucose pulses. Just after the addition of the substrate the IC-cells are relieved of any growth limitation. Probably the repeated sequence of this exogenous phase and the subsequent starvation phase has an influence on the substrate uptake behaviour of the bacteria; both *Arthrobacter* and *Sphaerotilus natans* manifested a larger overcapacity in the IC-chemostats.

Overcapacity means that the cells are maintained in a state in which they are always ready to take up substrates suddenly introduced in the culture. Energy has to be provided for keeping enzymes and control systems active. Maintaining the growth potential of the cells on a high level will decrease the growth efficiency and thus the cell yield. Conform to this hypothesis the dry weight, for all D-values, of the IC-cultures were slightly lower than for the CC-cultures. Also the continuous adaptation of the metabolism of the IC-cells and its control will result in a lower growth efficiency.

Another feature of the IC-cultures is the higher production of overflow metabolites (indicated in Tables 4 and 5 as COD). In the exogenous phase the substrate uptake rate is triggered towards high values, the growth rate not being able to switch to higher values (Kjeldgaard et al. 1958; Harvey 1970; Harrison and Topiwala 1974). Substrate is entering the cell and in order to circumvent the dangerous building up of substrates in the cell, they are channeled in the metabolic pathways for a short processing and then secreted. When no more stress is put on the cells the overflow metabolites can be taken up again. Another response of cells towards substrate sufficient media is the building of reserve polymers. *Sphaerotilus natans* is capable of producing poly-β-hydroxybutyric acid and *Arthrobacter* can accumulate polysaccharides (Zevenhuizen 1966). Upon starvation the micro-organisms can rely on these substances.

Figures 3 and 4 show that the q_{max} of the CC-cultures always remains below the maximal steady state uptake rate, corresponding to the μ_{max}. For IC-cultures this value is exceeded and the question can be asked wether in IC-cultures the μ_{max} cannot be increased.

It seems that the bacteria are adapting to intermittent feeding by increasing their maximal uptake rate. Apart from this, higher K_m values were found for the IC-cultures; the difference being higher for *Arthrobacter*. Studies (Umbarger 1969; Neijssel et al. 1975) indicate that certain species have more than one uptake system for a certain substrate. The uptake mechanism with the highest affinity (lowest K_m) is induced whenever the growth is limited by that particular substrate. In substrate sufficient cultures an uptake mechanism with low affinity is synthesized. This is an important tool in the regulation of metabolism. The difference found between IC- and CC-cultures in this study is not large enough to suppose an induction of a different uptake system. An adaptation of K_m for glucose has also been observed with *Corynebacterium* (Law et al. 1976).

Comparing the behaviour of *Arthrobacter* and *Sphaerotilus natans* it is clear from Figs. 3 and 4, that the overcapacity of *Arthrobacter* is larger than for *Sphaerotilus* especially in the IC-cultures. These observations have ecological consequences. Changing from a C-feeding to an I-feeding will have a larger effect on *Arthrobacter* than on *Sphaerotilus*. Growing together a reactive and a less reactive species in a continuous culture and changing from a C- to an I-feeding will result in the selection of the reactive species. It will be favoured because of its larger overcapacity, allowing it to take up the largest amount of substrate. This will ultimately result in a higher growth rate leading to its predominance in the population.

We feel that the curing of filamentous bulking by changing from a C- to an I-feeding can be explained in the same way. Activated sludge systems are open cultures and with a continuous supply of substrates, the system selects for cells with a relative high degree of energetic coupling and a low reactivity. If filamentous microorganisms belong to this group, sludge bulking can be explained. With an I-feeding or in a plug flow system, species with the highest reactivity are selected, capable of taking up the substrates rapidly during the exogenous phase. Species producing reserve substances are favoured over species producing over-flow metabolites. The larger uptake rate and an efficient metabolism of reserves will result in a larger overall growth rate and will lead to predominance in the activated sludge.

Conform with this hypothesis is the fact that sludges growing in I-fed or plug flow systems express a high substrate uptake rate and an elevated capacity for reserve polymer building. *Sphaerotilus natans* was shown to be a species with a low reactivity; no information about this property of other filamentous micro-organisms is available.

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Appendix 1

Accepting the Monod equation for growth (1), one can prove that the same relation yields for the substrate uptake (5)

$$\mu = \mu_{\max} \cdot \frac{S}{K_s + S}, \tag{1}$$

$$\mu = \frac{1}{X} \cdot \frac{dX}{dt}, \tag{2}$$

$$Y = -\frac{dX}{dS}. \tag{3}$$

Combing (2) and (3) yields

$$\frac{1}{X} \cdot \frac{dS}{dt} = -\frac{\mu}{Y}. \tag{4}$$

Substituting μ from eq. (1) gives

$$\frac{1}{X} \cdot \frac{dS}{dt} = -\frac{1}{Y} \cdot \mu_{\max} \cdot \frac{S}{K_s + S}. \tag{5}$$

Equation (5) confirms the enzyme mediated uptake of substrates. Peculiar about this mathematical derivation is the fact that it demonstrates that the K_s for growth and the half saturation constant for substrate uptake are one and the same. For steady state and balanced growth this statement can be accepted. For unbalanced growth and transients, as in activated sludge systems with a substrate gradient, uptake is no longer coupled to growth and two different constants have to be defined. K_s can be used for growth and K_m for substrate uptake rate (6)

$$q = q_{\max} \frac{S}{K_m + S}. \tag{6}$$

Key: μ is the growth rate; h^{-1}, Y is the biomass yield; g cells · g glucose^{-1}, X is the biomass concentration, S is the substrate concentration, K_s half saturation constant for growth, K_m half saturation constant for substrate uptake.

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